

IT IS CLAIMED:

Sub A 2/5

1. A method for testing the allergenicity of a heterologous protein produced by a plant or animal that has been genetically modified to produce that protein, comprising the steps of:

- (a) sensitizing a newborn dog from an atopic dog colony with a first extract prepared from tissue of the genetically modified plant or animal and containing a mixture of plant or animal proteins and the heterologous protein, by injecting, feeding or applying the extract to the skin of the newborn dog,
- 10 (b) after a period sufficient to allow the dog to establish an immune response to the sensitizing extract, challenging the dog with the extract,
- (c) observing the degree of allergic response provoked,
- 15 (d) if a detectable skin reaction is observed, comparing the degree of skin reaction observed with that observed by carrying out steps (a)-(c) above, but where the sensitizing step (a) or applying step (b) is carried out with a second plant or animal extract containing substantially the same proteins as the first extract but lacking the heterologous protein, and
- 20 (e) if the degree of skin reaction at (c) is greater than that observed by carrying out steps (a)-(c) in accordance with step (d), identifying the heterologous protein as a potential allergen in humans.

2. The method of claim 1, wherein said challenging and observing steps are selected from the group consisting of:

- (a) applying the allergen material to a skin region of the dog and observing a local wheal reaction at the application site as the allergic response (skin test);
- 25 (b) feeding the allergen material to the dog, and observing gastrointestinal upset as the allergic response (feeding test);
- (c) injecting the allergen material directly with the wall of the stomach of the dog and observing a local wheal reaction at the application site as the allergic response (gastroendoscopy test);
- 30 (d) administering the allergen material by inhalation to the dog, and observing bronchial constriction as the allergic response (inhalation test); and
- (e) applying the allergen material with a patch immobilized on the skin and observing inflammation at the site of application (transdermal patch test).

Sub B2
3. The method of Claim 1, wherein the extract is obtained from a transgenic plant.

Sub A5
4. The method of claim 3, wherein the plant is a crop plant selected from the group consisting of corn, barley, wheat, rice, peanut, sorghum, millet, spelt and soy.

Sub B3
5. The method of claim 1, wherein step (d) is carried out by applying the first extract to a dog sensitized with said second extract.

Sub A4
10 6. The method of claim 1, wherein substantially no skin reaction is observed in carrying out steps (a)-(c) in step (d).

Sub B4
15 7. The method of claim 1, wherein said extract is prepared by forming a tissue powder and extracting the powder with a selected extract medium.

Sub B5
20 8. The method of claim 1, which further includes, when a potential allergen is identified in step (e), repeating step (c) with the heterologous protein in purified form.

Sub A5
25 9. The method of claim 8, wherein the heterologous protein is produced by an organism other than the transgenic plant or animal.

Sub A5
10. The method of claim 8, wherein the heterologous protein is produced by the transgenic plant or animal.

Sub A6
25 11. The method of claim 1, which further includes, when a potential allergen is identified in step (e), separating proteins in the first extract and reacting the separated proteins with an immunoglobulin obtained from the dog sensitized with the same extract, to identify whether the protein that reacts with the immunoglobulin is the heterologous protein.

Sub A6
30 12. The method of claim 1, wherein the degree of skin reaction observed in step (c), compared with that observed in step (d) is indicative of the degree of allergenicity expected in humans.

13. A method for testing a biological substance for allergenicity in humans, comprising the steps of:

(a) sensitizing a newborn dog from an atopic dog colony with (a1) at least one known allergen in humans, (a2) a non-allergen control material, and (a3) a sample containing the test substance, by injecting or feeding the allergen, control material, and test substance to the dog,

(b) after a period sufficient to allow the dog to establish an immune response to the allergen:

(b1) confirming that said sensitizing has provoked an appropriate immune response in the dog by challenging the dog with the known allergen and observing an allergic response in the dog,

(b2) confirming that said sensitizing has not provoked an inappropriate immune response in the dog by challenging the dog with the control material and observing the absence of an allergic response in the dog, and

(b3) challenging the dog with the test substance and observing the degree of allergic response provoked or no response, and

(c) if an allergic response is observed in (b1) and (b3), but not (b2), identifying the test substance as a potential allergen in humans.

14. The method of claim 13, for use in grading the degree of allergic response produced by the test material, wherein step (a1) includes sensitizing the dog with at least two different allergens known to provoke a different degree of allergic response in humans, step (b1) includes challenging the dog with each of the at least two different known allergens, thus to determine the degree of immune response associated with the different known allergens, and in step (c) if an allergic response is observed in (b1) and (b3), but not (b2), matching the degree of response to the test allergen with one or more of the responses observed in step (b1).

15. The method of claim 14, wherein said known allergens include at least three allergens selected from the group consisting of peanut proteins, ragweed proteins, milk proteins, wheat proteins, and soy proteins.

16. A dog useful for testing a biological substance for allergenicity in humans, (i) obtained as a newborn from an atopic dog colony and

(ii) sensitized as a newborn with (a) at least one known allergen from humans, (b) a non-allergen control material, and (c) a sample containing the substance to be tested, by injecting the allergen, control material, and test substance into the dog.

5 17. The dog of claim 16, which is sensitized with at least two different allergens known to provoke a different degree of allergic response in humans.

10 18. The dog of claim 16, which is useful for testing allergens related to the known allergen, wherein the known allergen is a cereal, and the testing allergen is a cereal other than the known allergen.

15 19. The dog of claim 18, wherein the known allergen is a pollen, and the testing allergen is a pollen other than the known allergen.

20 20. The dog of claim 18, wherein the known allergen is a nut, and the testing allergen is a nut other than the known allergen.

20 21. A composition for use in sensitizing the dog of claim 16, which includes a mixture of peanut extract, ragweed extract, milk proteins, wheat proteins, and soy proteins, in a weight/volume ratio of about 1:1:1:1:1.

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